The Pseudopodia of the Leucocytes of Invertebrates.

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With Plates 1 and 2.

It is to Wharton Jones that we owe the first comprehensive account of the leucocytes in the various groups of Invertebrata: in an important paper, published in 1846 (**6**), he described and figured them as of "stellate appearance," and sometimes as shooting out "cilia-like processes." Since then numberless authors have studied these cells and pictured them as provided with free outstanding pseudopodia. Evidence is brought forward in this paper that the spiny appearance of leucocytes is of the nature of an optical illusion or due to changes taking place under abnormal conditions, that freely projecting fine processes are seldom, if ever, produced in the fluids of living invertebrates, and that the pointed pseudopodia so often figured are merely the optical sections of delicate, more or less folded films.

Various authors have from time to time mentioned the flattened expanded shape of the processes of the leucocytes of the invertebrates, as, for instance, Cattaneo (**1**) and Dekhuysen (**2**), referred to below; but none seem to have realised that this is the normal shape of the pseudopodia. In 1898 (**4**) I drew attention to the presence of membranous expansions of the leucocytes in the Polychæte worm Glycera, and at that time observed the same structures in other invertebrates; but other work prevented my pursuing the subject, and it was not till last winter that it was again taken up, when I had the opportunity of confirming and extending my observations at the Marine Biological Laboratory in Plymouth.

No attempt will here be made to describe in detail the various kinds of corpuscles found in the blood or cœlomic fluid, nor will the present paper deal with their granular contents. These subjects have been dealt with by many writers, more especially by Cuénot and by Kollman, who gives a very complete bibliography in his excellent memoir (**8**).

As examples of the Annelida we may take the Polychæte Arenicola and the Oligochæte Lumbricus. The cœlomic fluid of Arenicola contains abundant rounded and oval corpuscles, with others of intermediate form. At first sight the rounded cells seem to be provided with numerous pointed processes projecting in all directions; but more careful examination under the oil-immersion lens reveals the fact that these "pseudopodia" are in reality but optical sections of membranous expansions of cytoplasm thinning out peripherally to a very delicate and almost invisible film (Pl. 1, figs. 2 A and B). Here and there the membrane is strengthened by a rib, which appears as an outstanding process under a lower power.

The cells floating in the cœlomic fluid of Lumbricus, so often figured as provided with fine, frequently branching processes (Metchnikoff [**9**], Keng [**7**]), can, if examined quite fresh, be seen to throw out very delicate membranes. These, being somewhat folded and crinkled, give the appearance in optical section of long, branching pseudopodia (Pl. 1, figs. 3 A, B, C). The membrane of the leucocyte of the earthworm is thinner than that of Arenicola, hence the processes seem to be more delicate.

It is difficult to decide whether several independent membranes are produced, or whether the various lobes often seen passing out from the cell in different planes are really formed by the complicated folding of a single sheet of cytoplasm extending originally all round its edge. In Arenicola it is usually of the latter structure (Pl. 1, figs. 1 A, B), and a favourite attitude is that shown in Pl. 1, fig. 2 A, where the cell and its membrane somewhat resemble a half-opened flower. On the other hand, in the earthworm the membrane in freely floating cells is much folded and seems to spring from any point on the surface (Pl. 1, fig. 3 B). Frequently in Lumbricus, as well as in Arenicola, there appear to be several separate outgrowths.

Very similar to the leucocytes of the Annelids are the cells floating in the blood of the Mollusca. In Pl. 1, figs. 5, 6 and 7 of the leucocytes of Ostrea and Mytilus show the extensive membranes, the thickenings and folds of which yield the deceptive appearance of delicate pseudopodia.

As an example of the Arthropoda we may take the shore crab, Carcinus mænas. Its blood contains rounded, granular corpuscles, which may produce a large process at one side only (Pl. 1, fig. 9 B), or various processes projecting in all directions and thinning out to delicate films bearing many sharp points (Pl. 1, fig. 9 A).

The Echinoderms have long been known to possess very "amœboid" cœlomic corpuscles. In his interesting paper on "plasmodium" formation (3) Geddes figures the leucocytes of Echinus as provided with very long, branching pseudopodia, some of which join so as to encircle a space. In reality no such processes are present in fresh corpuscles, and they, like the ring-like arches, are merely representations of optical sections of an extensive membrane folded with rounded surfaces (Pl. 2, fig. 11). Very soon, however, after the cœlomic fluid has been removed from the animal the membrane begins to stretch out wing-like films in various directions, and these soon acquire a jagged edge with sharp points (Pl. 2, fig. 12).

In the above brief review have been selected from the various groups only a few typical examples out of the large number of genera examined. They are sufficient to show that the formation of delicate lamellæ of cytoplasm is characteristic of the leucocytes of all the invertebrate Cœlomata. When the cells are freely floating in a hanging drop the membranes are seen to be motile, continually undergoing change of shape, and capable of being more or less completely withdrawn. This motility varies considerably: in some cases they can be observed to wave about quite quickly, while in others they seem almost still. Whenever they come into contact with a foreign object, as, for instance, a glass slide or a cover-slip, the membranes tend to cling to it and spread over its surface as a thin film, the edge of which extends like a drop of oil on water. This well-known habit, observed by Metchnikoff (**9**) and many others, is doubtless related to changes of surface tension, and is of great importance to the animal in dealing with intrusive foreign particles and parasites entering the body-fluids. Though marked in Annelids and Molluscs (Pl. 1, fig. 5), it is still more pronounced in Crustaceans and Echinoderms, leading most of the cells to flatten themselves as a mere film over a glass surface. As shown by Theel (**12**), a piece of cover-slip introduced into a living starfish will very soon become almost completely covered and can then be conveniently stained and mounted (Pl. 2, fig. 14). Tait has recently suggested the name "thigmocyte" for these cells in the Crustacea (**11**).

Moreover, it has long been known that the body-fluids of the Invertebrata rarely clot (as in the Vertebrata and some Crustacea) by fibrin formation, but more usually by the gathering together of the leucocytes in irregular clumps and strands. These are the so-called "plasmodia" first described in detail in Echinus by Geddes (**3**), who thought the leucocytes became completely fused and formed a mass comparable to the plasmodia of Myxomycetes. But I agree with Michel (**10**) that the cells do not really lose their identity, are merely connected by their hyaline ectoplasmic layer, and are capable of resuming their independence under certain conditions. That this very marked tendency to form such agglutinations is due to the presence of the membranous extensions described above there can be little doubt, and that it is a most useful property is due to the fact that it serves not only to close wounds, but also to surround foreign particles or parasitic organisms too large to be dealt with by a single leucocyte (**3**, **5**).

It now remains to be proved, firstly that the membranous pseudopodia are not artefacts, are not due to contact with foreign bodies, and are present in the expanded condition on cells floating in the fluids of the living animal; and secondly, that the delicate radiating pseudopodia sometimes seen are not normally developed in these fluids, and are only formed under abnormal conditions. For this purpose a technique must be adopted which will enable us to fix the leucocytes rapidly in their normal state, without shrinking the membranes beyond recognition. Having tested many of the well-known fixatives, such as osmic acid, Flemming's fluid, Bouin's fluid, corrosive acetic, etc., I find the only reagent giving a satisfactory result is a watery solution of iodine. A strong solution of iodine in potassium iodide may be used, diluted to about the colour of sherry with normal saline for terrestrial and freshwater animals, and with sea-water for marine forms. If desired it can be followed by Bouin's fluid, and the cells stained and mounted in balsam. Such a solution of iodine introduced under the cover-slip fixes the finest pseudopodium or the most delicate protoplasmic film with marvellous rapidity and precision; and fluid dropped straight into the solution from the animal displays the leucocytes fixed in their natural condition.

Now if this test be applied to the cœlomic fluid of Arenicola the vast majority of the leucocytes are fixed with a wide sheet of protoplasm extending all round, and giving them somewhat the appearance of an open umbrella, as shown in Pl. 1, figs. 1 A and B. Although most of the rounded cells are of this type, there are many with more or less pronounced ridges or extended films projecting from the cell-body in various directions (Pl. 1, fig. 1 c). Again, in the earthworm the leucocytes are likewise found fixed with membranous extensions irregularly lobed and sometimes of relatively enormous size (Pl. 1, figs. 4 A and B). Similarly in the blood of Molluscs the membranes are seen to be more or less completely extended (Pl. 1, fig. 6). Most striking of all, perhaps, is the case of the Echinoderm. In Asterias, for example, all the leucocytes display a much-folded membrane, bulging in a characteristic way so as to give them a vesicular appearance (Pl. 2, figs. 11 A–D); but so far as I have been able to make out the vesicles are not really closed, but always have an opening bounded by the thin free edge of the membrane. On the other hand, the blood of Crustacea dropped into iodine solution seems to show that in them the membranes are not so much expanded; for although often quite recognisable in spite of the abundant coagulum they are generally short, and may be represented merely by blunt pseudopodia—at all events in the species I have studied (Carcinus mænas, Eupagurus prideauxii, Astacus fluviatilis).

It is quite clear, then, that in most invertebrates the membranous pseudopodia of the leucocytes are normally expanded in the living animal, and it is equally clear that these cells are not provided with fine outstanding pseudopodia. Although not prepared to assert that such processes never exist in the normal body-fluids, I can state that they were not found in preparations of freshly-drawn material. Nevertheless they undoubtedly rapidly make an appearance in blood or cœlomic fluid allowed to stand, and may often be of extraordinary length and tenuity (Pl. 2, figs. 13 A, B). Leucocytes floating in a hanging drop may thus come to resemble delicate Heliozoa with fine processes radiating all round them. The formation of such fine pseudopodia is almost certainly abnormal, and due to physico-chemical changes taking place in the fluid, and possibly a sign of approaching death. As to their mode of origin, it seems probable that they are not spun out as free-growing threads, but are rather the remnants of previously existing membranes, being the thicker ridges or supporting folds which remain when the intervening film has thinned out or retracted. The appearance of certain "thigmocytes," such as that shown in Pl. 1, fig. 8, strongly suggests this interpretation, and the dentated edge of the membranous folds so frequently seen (figs. 9 A and 12) may represent initial stages in the production of pointed pseudopodia. The leucocytes of Arenicola figured by Dekhuysen appear to be in this condition (**2**).

The chief results recorded in this paper may be briefly summarised as follows: The leucocytes of the blood or cœlomic fluid of the invertebrate Cœlomata are provided with more or less extensive membranous processes of cytoplasm. The freely-projecting pseudopodia usually described are either figured from optical sections of the folded membranes or from cells which have produced them under abnormal conditions. These fine pseudopodia may be present on cells in fluid withdrawn from the body and which has been allowed to stand, and are probably derived from pre-existing membranes. The delicate motile membranous folds are usually expanded in the normal fluids of the living animal.

References.

1. Cattaneo, G.—"Sugli 'Amebocite' dei Crustacei," 'Zool. Anz.,' Bd. xi, 1888.

2. Dekhuysen, M. C.—"Ueber die Thrombocyten," 'Anat. Anz.,' Bd. xix, 1901.

3. Geddes, P.—"Observations sur le fluide périviscéral des Oursins," 'Arch. Zool. Expér.,' t. viii, 1880.

4. Goodrich, E. S.—"On the Nephridia of the Polychæta: Part II. Glycera and Gonida," 'Quart. Journ. Micr. Sci.,' vol. 43, 1898.

5. ——— and Pixell Goodrich, H.—'Leucocytes and Protozoa.' Sir W. Osler's volume, 1919.

6. Jones, T. Wharton.—"The Blood-corpuscle considered in its Different Phases of Development in the Animal Series," 'Phil. Trans. Roy. Soc.,' vol. cxxxvi, 1846.

7. Keng, Lim Boom.—"On the Cœlomic Fluid of Lumbricus terrestris," ibid., vol. clxxvi, 1895.

8. Kollmann, M.—"Recherches sur les Leucocytes," 'Ann. Sc. Nat. Zool.,' ser. 9, t. viii, 1908.

9. Metchnikoff, E.—'Leçons sur la pathologie comparée de l'inflammation,' 1892.

10. Michel, A.—"Sur la prétendue fusion des cellules lymphatiques en plasmodes," 'C.R. Acad. Sci. Paris,' t. cvi, 1888.

11. Tait, J.—"Capillary Phenomena Observed in Blood-cells," 'Quart. Journ. Exper. Physiol.,' vol. xii, 1918.

12. Theel, H.—"Remarks on the Activity of Amœboid Cells in the Echinoderms," 'Festschrift f. Lilljeborg, Upsala,' 1896.

EXPLANATION OF PLATES 1 and 2,

Illustrating Mr. Edwin S. Goodrich's paper on "The Pseudopodia of the Leucocytes of Invertebrates."

[All the figures, except fig. 2, have been drawn with the camera lucida at a magnification of 1600 diameters.]

PLATE 1.

Figs. 1 A, B and C.—Three cœlomic corpuscles of Arenicola grubei, fixed in iodine. B, in profile; C shows membranes projecting in several planes.

Figs. 2 A and B.—Cœlomic corpuscles of Arenicola ecaudata, drawn from the living. × 1600.

Figs. 3 A, B and C.—Cœlomic corpuscles of Lumbricus herculæus, drawn from the living.

Figs. 4 A and B.—Two cœlomic corpuscles of Lumbricus, fixed in iodine.

Fig. 5.—Blood-corpuscle of Ostrea edulis, much flattened on a cover-slip; from the living.

Fig. 6.—Blood-corpuscle of Ostrea edulis, fixed in iodine.

Fig. 7.—Blood-corpuscle of Mytilus edulis, from the living.

Fig. 8.—Blood-corpuscle of Carcinus mænas, much flattened on a cover-slip and fixed in iodine.

Figs. 9 A and B.—Blood-corpuscles of Carcinus mænas, from the living. A shows two corpuscles agglutinated and floating; B, a single corpuscle adhering to glass.

PLATE 2.

Fig. 10.—Cœlomic corpuscle of Asterias glacialis, fixed in iodine.

Figs. 11 A, B, C and D.—Cœlomic corpuscles of Asterias glacialis, fixed in iodine and Bouin's fluid, stained, and mounted in balsam.

Fig. 12.—Cœlomic corpuscle of Asterias glacialis in a hanging drop; from the living.

Figs. 13 A and B.—Cœlomic corpuscles of Asterias glacialis, flattened out on a slide; drawn from the living.

Fig. 14.—Two agglutinated cœlomic corpuscles drawn from a cover-slip inserted into an Asterias glacialis and fixed with iodine.